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PARTIAL CHARACTERIZATION OF AROMA PRODUCED BY SUBMERGED CULTURE OF MOREL MUSHROOM MYCELIUM.

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ABSTRACT - Among the edible mushrooms, the genus *Morchella* presents the advantage to produce pleasant odorous substances by mycelial biomass as well as by fruiting bodies. Three morel mushroom mycelium were grown in submerged culture in order to approach the optimal conditions for biomass and aroma production. Succession of odorous substances, as fruity, woody-musty and flowery notes were expessed during incubation on malt extract broth. Sniffing evaluation allowed to select *Morchella crassipes* for further cultivation on ammonium-glucose basal medium and ammonium-glucose malt extract medium and aroma production. Samples of mycelial biomass and fresh or rehydrated fruiting bodies were extracted by distillation-extraction with methylene chloride as solvent. The extracts obtained were concentrated under nitrogen flux and analysed by gas chromatography. In parallel, an excess load of 5 standard aromatic molecules supposed to be contained in the mushroom extracts were added to the samples before analysis. Chromatographic profiles analysis show that similarities between mycelium extracts are higher than between mycelium and fruiting bodies extracts. They also reveal the losses of many molecules for the extracts of rehydrated material , indicated by the absence of many peaks with low retention time. 1-octen,3-ol amounts are shown to be higher in mycelium than in fruiting bodies.

RÉSUMÉ - Il existe aujourd'hui des champignons "nobles" pour lesquels la maîtrise du développement en vue de l'obtention de volumes importants de carpophores n'a pu être efficacement établie *in vitro*. Certains, cependant, présentent la particularité de posséder un mycélium aromatique, mais rares sont les espèces dont l'odeur du mycélium rappelle celle du carpophore. La morille, en particulier, présente cette caractéristique. Il est donc apparu intéressant d'en cultiver le mycélium afin de caractériser l'arôme produit. Au cours d'une étape de sélection, les cultures sont réalisées sur un milieu à l'extrait de malt. Croissance mycélienne et pH des milieux sont relevés sur 14 jours, en parallèle avec l'intensité et la qualité des arômes évalués par flairage. Des fractions mycéliennes retenues pour l'opération d'extraction et des échantillons de carpophore sont homogénéisés mécaniquement, soumis aux ultrasons, puis traités par co-distillation au dichlorométhane sur un appareil de Lickens-Nickerson pendant 40 minutes. Les extraits sont ensuite concentrés 5 fois sous courant d'azote et dosés en C.P.G. Ramenées à la même quantité de biomasse, les concentrations en extraits aromatiques totaux dans les fractions mycéliennes et les carpophores sont équivalentes. On note que le milieu de culture influence la composition de l'arôme extrait du mycélium. En utilisant la méthode des surcharges ■ moyen de quelques molécules standards, on montre cependant que le mycélium est plus riche en octène-1, ol-3 que le carpophore.

INTRODUCTION

Few edible mushrooms have been integrated in cultivation processes which allow to produce important quantities of fruiting bodies. Nevertheless, many mushrooms exhibit aromatic mycelia, but rare are those which have odorous qualities identical to their corresponding fruiting bodies (Babcock, 1939). The morel mushroom presents this particular feature (Gilbert, 1960), and the cultivation of its mycelium appears susceptible to conduct to an appreciable aromatic food additive.

From the 50's, the researches have mainly been concerned with submerged production of morel mycelium, a patent was deposited by Szuecs (1956). The product was generally washed, dehydrated and crushed to give a powder used for incorporation in food preparations, as soups.

In view to optimize the growth of the mycelium and to reduce the production costs, various solutions have been described for the formulations of the culture media. Several fermentation processes have been proposed utilizing synthetic substrates (Willam *et al.*, 1956), by-products of the food industry (Gilbert, 1960; Litchfield *et al.*, 1963; Litchfield, 1967) or residues of the paper industry (Le Duy *et al.*, 1974).

Comparatively to the works devoted to improve the mycelium production, very few studies have been focused to the separation of the aroma fraction from the mycelium (Szuecs, 1950). In fact, volatile coumpounds extracted from fruiting bodies were described by Pyysalo (1976) from seven species and by Audouin *et al.* (1989) from four species, including a morel.

The influences of the culture conditions on the flavour qualities of the mycelium were reported for fungi by Sanchez-Font *et al.* (1985) and reviewed for basidiomycetes by Gross and Asther (1989) and Gallois *et al.* (1990). The general way to optimize the aroma production in submerged fermentation has been reviewed by Belin *et al.* (1992).

The production of an aromatic mycelium extract allowed to valorize various by-products and have, independantly of the seasonal conditions, the advantage to utilize the filamentous phase of a mushroom in place of the fruiting body which remains an appreciable food.

MATERIAL AND METHODS

Organisms and maintenance

Three mycelia of *Morchella* were provided from the collection of Station d'Agronomie et de Mycologie (I.N.R.A., Clermont-Ferrand, France). *M. crassipes* MCR 92.24 (CBS N° 289.63, Baarns), *M. esculenta* MES 91.9 (from danish forest) and *M. hortensis* MH 88.7 (from Provence). The stains were stored at +4°C on potato dextrose agar slants (Bio-Mérieux, Charbonnières-les-Bains).

Fruiting bodies of *Morchella sp.* fresh or dehydrated were collected for comparative studies.

Media, culture conditions and sensory analysis.

Incubations for growth kinetics and sensory analysis were performed on 6 ml of malt extract broth in test tubes carried by a slow speed inclined rotative holder at 25°C during 14 days. Each day, tubes were sampled in triplicate to minimize aberrant results. pH of the medium and biomass increase (dry matter) were measured. Qualitative identification of aroma production by sniffing was evaluated during the 14 days cultures. Aroma intensity was recorded versus an increasing scale: I (low) to III (high).

Aroma production was performed in 500 ml flasks containing 200 ml of liquid medium supplemented with chloramphenicol (50 ppm, Aldrich). The liquid cultures were run on a basal (B) medium containing glucose (20 g/l, Aldrich, Strasbourg, France), ammonium-monophosphate (2 g/l, Aldrich) and 100 ml/l of the Szuecs's mineral solution and on a basal malt extract medium (BME) consisting of the same B medium enriched by malt extract broth (10g/l; BioMérieux, France). Initial pH was adjusted to 5.9 for all cultures. Szuecs's mineral solution include, in g/l: $CaCO_3$: 63; $MgSO_4,7H_2O$: 15,3; K_2SO_4: 11; $MnSO_4,4H_2O$: 0,42; $Fe_2(SO_4)_3$: 0,086 (Szuecs, 1956). Incubation was run on a rotary shaker at 110 rev./min at 25°C during 14 days.

Preparation of the biomass extracts

Wet mycelia (25 g) and fresh or rehydrated fruiting bodies (12 g) were separately transfered in equal volume of distilled water and crushed during 45 sec with an Ultra-Turrax mixer (BioBlock, Strasbourg, France). Ultrasonication 20 sec, three times at 250 watts, using a Vibra Cell Sonics and Material apparatus (Danbury, CT, USA) 250 watts; BioBlock, France) was realized to burst the cell walls. The treatment was controlled by microscopic examination.

Aroma extraction and analysis

The aroma compounds were extracted from the crushed biomass by distillation-extraction in a Likens-Nickerson apparatus modified by Godefroot *et al.*, (1981), according to the work of Vidal *et al.*, (1988) on *Marasmus oreades*. Samples (10 ml) were distillated simultaneously with methylene chloride (20 ml) at 115 and 70°C, respectively, for 40 min. The organic phase was 5-fold concentrated (Vidal *et al.*, 1988; Audouin *et al.*, 1989) under nitrogen flux and quantified by gas chromatography. 0.1 ml of internal standart, g-undecanoic acid lactone (g-C11; 0.002 mg/ml in methylene chloride), was added to samples before extraction.

Chromatographic analysis were carried out on ▪ Spirawax column (internal diameter, 0.32 mm, and film thickness, 0.25 mm; Spiral, Dijon, France) using ▪ Packard chromatograph, model 627A (Chrompack, Middelburg, The Nederlands), equipped with a flame ionization detector. The oven rise temperature was set from 40 to 230°C, with a rate of 2°C/min. Injector and detector temperatures were 200 and 300°C respectively. Nitrogen (4 ml/min) was used as gas vector.

To identify some molecules in the morel mushroom extract by overloading, similar extraction and analysis of the samples were run after addition of 0.1 ml of ▪ standard solution in methylene chloride containing five aroma substances of known concentrations: 1-octen,3-ol (mushroom aroma), 2-heptanone (blue cheese aroma), 2,5-

dimethylpyrazine (nusty-roasty aroma), linalool (flowery terpenic odor) and acetophenone (almond aroma).

RESULTS AND DISCUSSION

Comparative study of the odorous notes of the entire cultures of the three *Morchella* strains.

The sniffing of the three cultures during the 14 days has shown some similarities in the aromatic profiles (Figure 1). Succession of dominant aromatic notes indicate: fruity notes in the beginning of the cultures, woody and nusty notes and finally, flowery notes. *M. esculenta* exhibited ■ particularity with an intercalation of a strong mushroom odor between the 7th and 9th days, just before the production of woody and nusty notes.

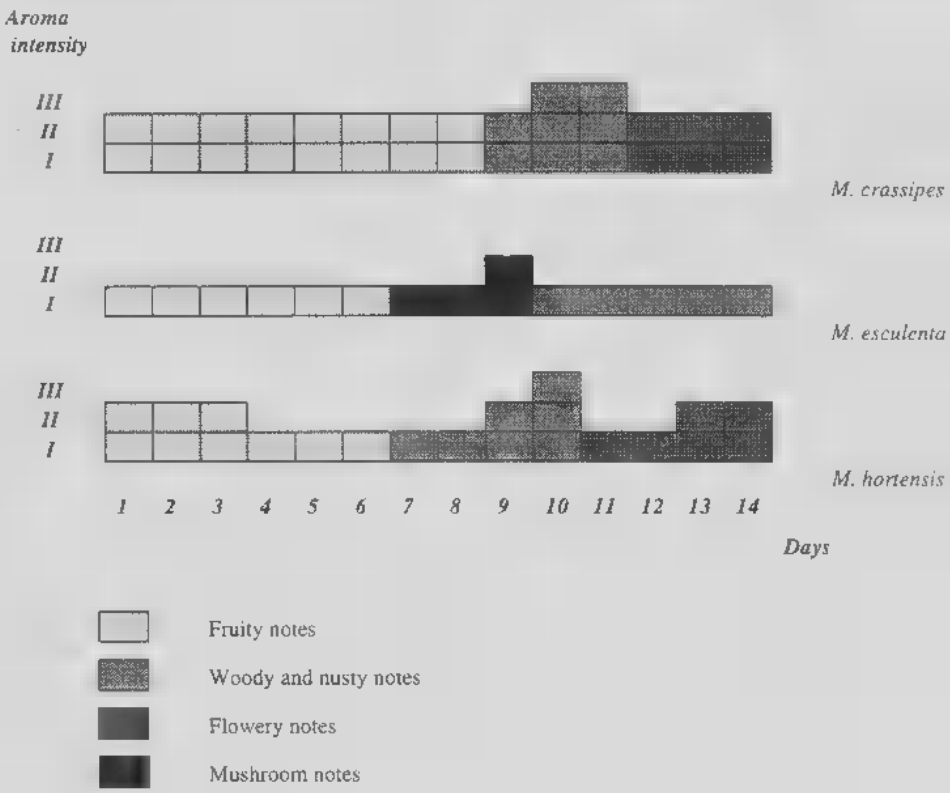


Figure 1.
Odorous profiles of submerged cultures of morel mushroom mycelium in malt extract broth.
Profils odorants des cultures mycéliennes de morille en bouillon à l'extrait de malt.

Morchella crassipes produced the higher and more stable aromatic intensity of all notes during the incubation on malt extract broth , so it was selected for aroma production.

Nevertheless, it has to be stressed that, in the same period, *M. crassipes* produced less biomass than the other strains; about 30% lower at the 5 last days of cultivation (Figure 2). This can be related to the pH conditions of the medium. The pH of the *M. crassipes* culture (Figure 3) remains in a more acidic zone (5,1 - 5,3), which is one or two points lower than the pH conditions favourable to the mycelium production as previuosly described (pH=6: Szuecs, 1956; Martin, 1981; pH=6,93: Willam *et al.*, 1956).

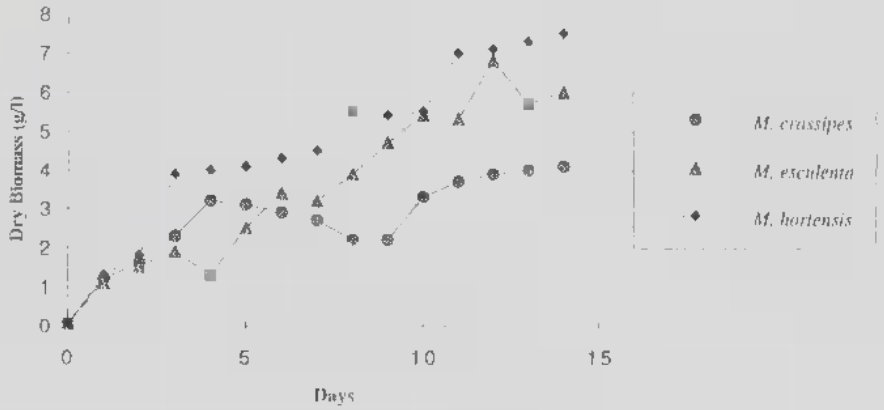


Figure 2.
Growth kinetics of three morel mycelia species.
Cinétiques de croissance du mycélium de trois espèces de morille.

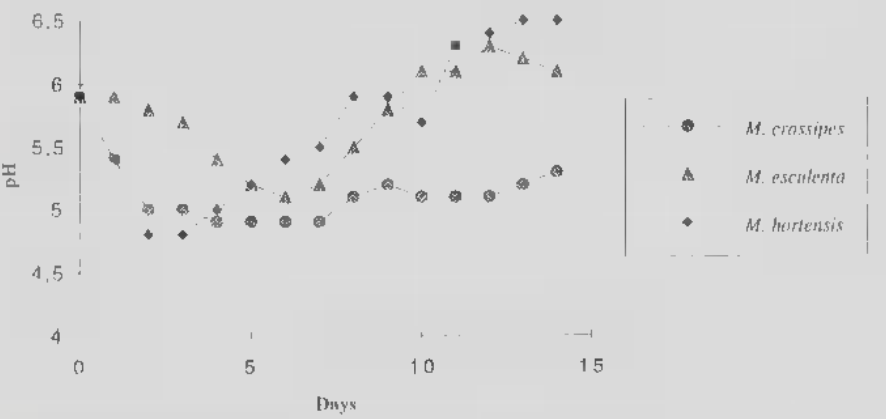
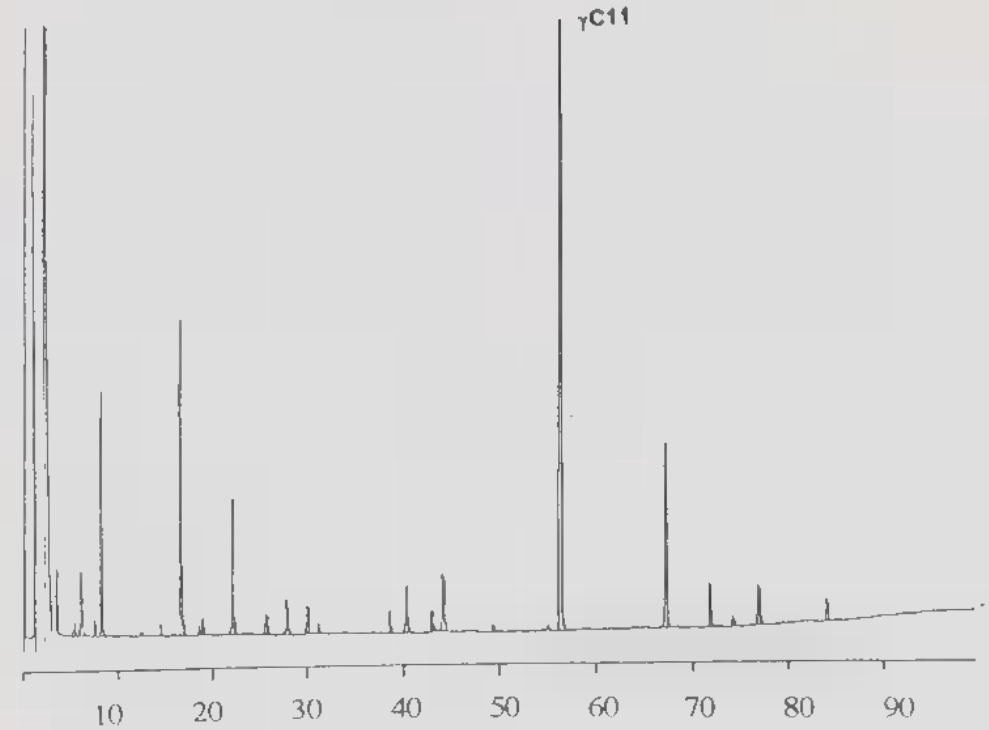
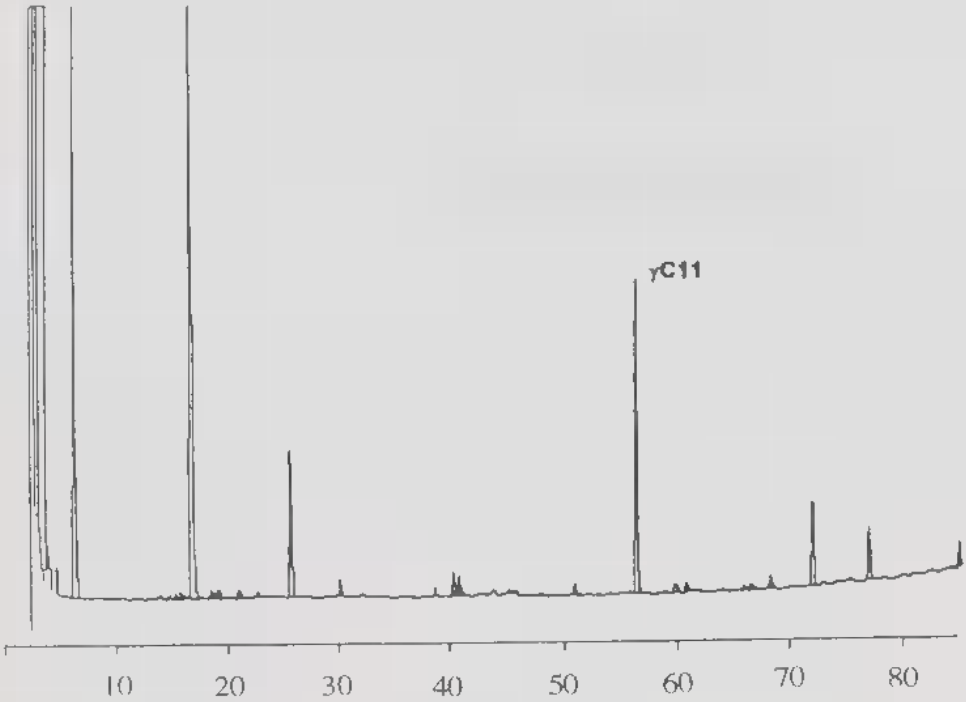


Figure 3.
pH variation in submerged cultures of three morel mushroom mycelia.
Evolution du pH dans les cultures en milieu liquide de trois espèces de morille.





Aroma production by Morchella crassipes.

Litchfield *et al.* (1963) have pointed out the positive role of glucose and ammonium-monophosphate on the growth of morel mushroom mycelium in submerged conditions.

Complementary, in this study, in which a similar basal medium was used, it has been observed that morel aroma was strong and perceptible during the first 12 days of cultivation and was not increased by a supplementation of organic nutriment, as malt extract, in the culture medium.

Chromatographic analysis performed on the mycelial extracts from the B medium (Figure 4) and the BME medium (Figure 5) are presented and compared to the results obtained from the fruiting bodies of fresh (Figure 6) or rehydrated (Figure 7) material.

On the basis of the profiles appearing on the chromatograms, a clear separation is visible between the mycelial extracts and the fruiting bodies extracts, the latter showing more important peaks with high retention time.

The presence of g-undecanoic acid lactone (g-C11) as an internal standart in the samples allowed to calculate two parameters: the extraction yield (YE) and the concentration of the total aroma (CT). The results confirmed that, even the extraction yield is lower (YE= 0,44; Table 1), the culture on a basal medium containing ammonium mono-phosphate and glucose is more favourable to the production of *Morchella* aroma (CT=170 ppm) in submerged culture.

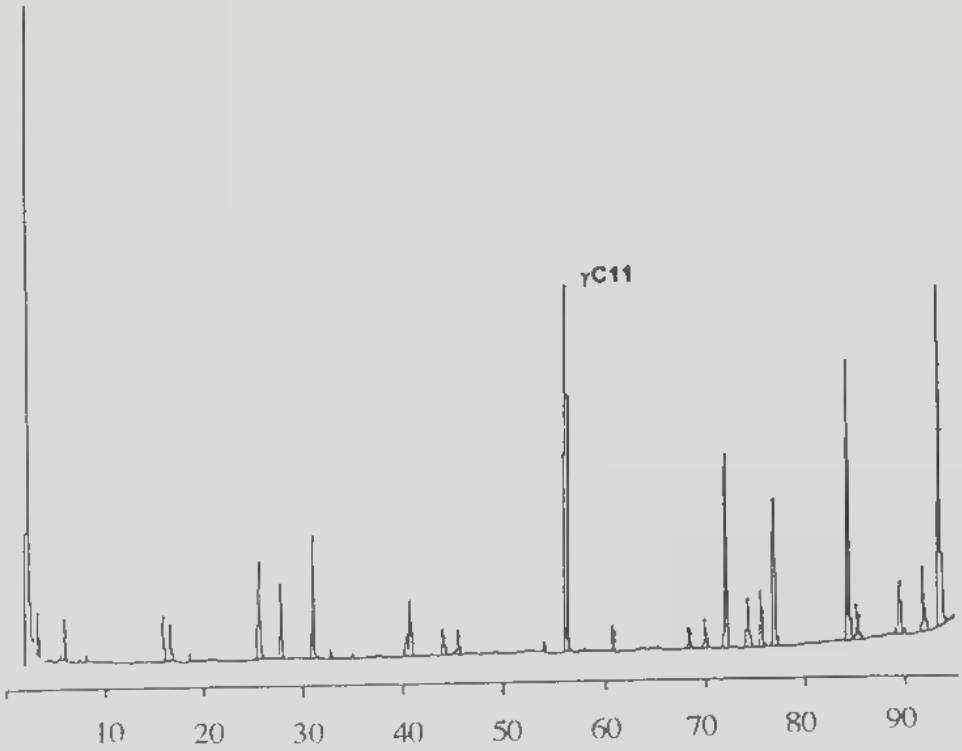
Table 1 shows also the results of a five molecules overloading of pure compounds in extracts taken from mycelium and fruiting bodies.

The 1-octen,3-ol appears more concentrated in the extracts issued from the mycelium (13-40 ppm) than in the extracts of the fruiting bodies (1-3 ppm). Nevertheless, these amounts remain very low. Utilizing a gassed stirred tank reactor to cultivate *Morchella esculenta*, Schindler and Seipenbush (1990) indicated that the growth form of the mycelium in submerged culture is of a great importance upon the formation of 1-octen,3-ol. For optimal formation of this molecule (70 ppm), small and compact pellets are preferable to loose pellets. The amount of 1-octen,3-ol can be greatly incresed if mycelium is submitted to shear stress and disruption (Schindler and Seipenbush, 1990), in the presence of linoleic acid as flavour precursor (Grosh and Wurzenberger, 1985; Bensoussan *et al.*, 1988; Muguet *et al.*, 1994).

The detection of small quantity of linalool (5 ppm) in mycelium cultivated on malt extract broth could explain the fruity notes detected by sniffing (Collins and Morgan, 1970).

Figure 4.
Chromatogram of extract of morel mycelium cultivated on ammonium-glucose basal medium.
Chromatogramme d'un extrait mycélien de morille cultivée sur milieu de base ammonium-glucose.

Figure 5.
Chromatogram of extract of morel mycelium cultivated on malt extract basal medium.
Chromatogramme d'un extrait mycélien de morille cultivée sur milieu de base à l'extrait de malt.



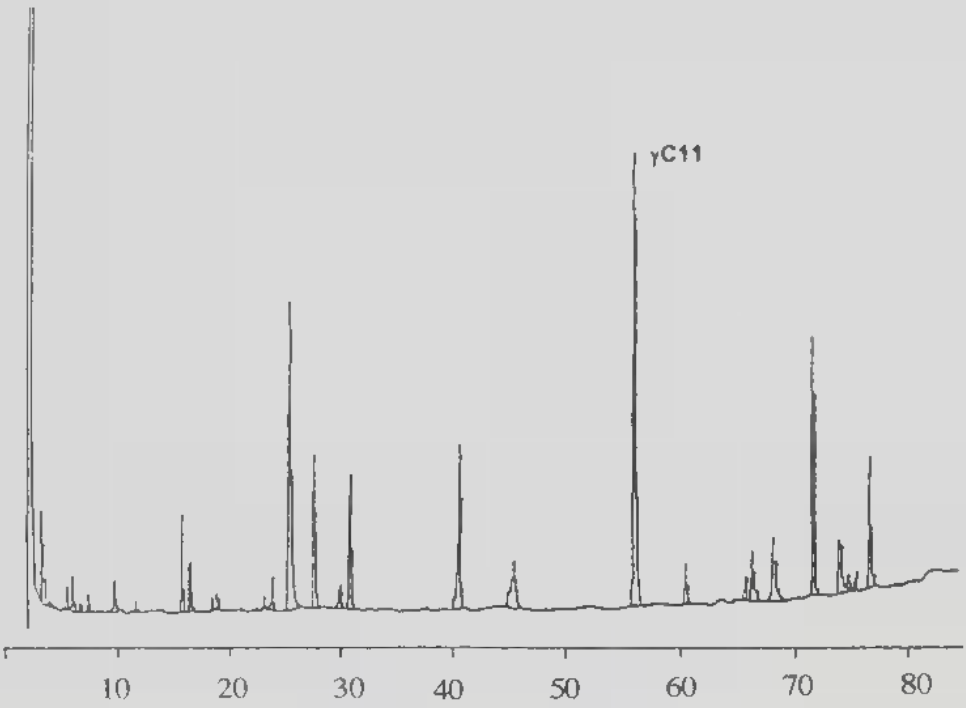


Table 1.
Identification of some volatile compounds extracted from morel mushroom aroma.
Identification de quelques composés volatils extraits de l'arôme de morille.

	SAMPLES			
	MYCELIUM		FRUITING BODIES	
	Glucose-ammonium	Glucose-ammonium malt extract	Fresh	Rehydrated
Extraction yield	0,44	0,65	0,56	0,57
Concentration of total aroma (µg/g):	153	69	70	170
Amount of identified molecules in the total aroma (µg/g):				
1-octene,3-ol	*40*	*13*	*1*	*3*
2-heptanone	*0*	*0*	*0*	*0*
2,5-dimethylpyrazine	*0*	*0*	*traces*	*5*
linalool	*0*	*5*	*0*	*0*
acetophenone	*17*	*1*	*4*	*29*

A comparative analysis of the chromatograms corresponding to the two fruiting bodies (Figures 6 and 7) show that the extracts of fresh material are qualitatively and quantitatively richer in volatile compounds than the dry material.

According to Maga (1981), the drying process promote the desorption of many volatile compounds. In this study, the loss has an important effect on the molecules of which retention times are lower than those of the g-undecanoic acid lactone; its peack is placed in the central part of the chromatograms.

CONCLUSION

We have demonstrated that aroma produced by morel mushroom mycelia are dependent on the strains and on the culture conditions. In a screening on malt extract broth, we have noticed alternation of odorous notes during the incubation, with, in the beginning, permanence of fruity notes.

The submerged conditions allow to grow mycelium of *Morchella* which amount of total aroma is in the same order with the quantities extracted from fruiting bodies (CT: 70-170 ppm).

Figure 6.
Chromatogram of extract of fresh morel fruiting body.
Chromatogramme d'un extrait de carpophore frais de morille .

Figure 7.
Chromatogram of extract of rehydrated morel fruiting body.
Chromatogramme d'un extrait de carpophore rehydraté de morille .

In comparison to fruiting bodies fresh or rehydrated, the mycelium appears to contain greater quantity of 1-octen,3-ol.

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